

CVIII. THE EXCRETION OF PURINE DERIVATIVES IN DOGS.

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It is generally assumed as a fact that allantoin is the terminal product of purine metabolism in dogs and that allantoin excretion in dogs has the same significance as uric acid excretion in man.

The difference between the purine metabolism in dogs and in man is believed to depend upon the presence in the dog's liver of the enzyme uricase, which oxidises uric acid to allantoin, and the lack of this enzyme in the human liver and in other human tissues [Wiechowski, 1907, 1908].

According to this conception the allantoin excretion in dogs and the uric acid excretion in man ought to have the same relative magnitude, if the only difference in their purine metabolism were that the terminal product in man was uric acid and in dogs allantoin.

It appears however from the literature concerning determination of allantoin in urines of dogs, that the excretion in many dogs is very large compared with the excretion of uric acid in man.

In papers by Wiechowski [1907, 1908], Schittenhelm [1909] and Hirokawa [1910] numerous determinations of the allantoin excretion in dogs are found. If the allantoin nitrogen in percentage of total N is calculated from their tables, it is evident that this percentage is very high compared with the uric acid N in percentage of total N in man. The percentage varies between 7 and 11.

Hunter, Givens and Guion [1914] point out this high ratio of allantoin to the total N excretion and emphasise that the allantoin output under ordinary conditions of diet or even in starvation may be so great as to account for nearly one-tenth of all the nitrogen excreted.

In man the excretion of uric acid N in percentage of total N is much smaller, the average being about 1 % if on purine-free diet.

The amount of uric acid excreted by dogs is almost always very small and the amount of other purine bases in the urine is insignificant.

In Dalmatian dogs, however, S. R. Benedict [1917] found a quite considerable excretion of uric acid, a finding which is confirmed by Wells [1918], Onslow [1923] and by Folin, Berglund and Derick [1924].

Onslow [1923] studied the inheritance of this anomaly in two generations of offspring from a Dalmatian and a terrier, and found that the anomaly was inherited according to the Mendelian law.

In his papers, however, attention was directed only to the uric acid excretion. A series of allantoin estimations was made, but no remark was passed about the very large excretion of allantoin in all the dogs, which according to the tables in many dogs amounts to more than 20 % of the total N.

When this very large excretion of allantoin in dogs is compared with the uric acid excretion in man, it is evident that the purine metabolism in the dog and in man must be quite different not only as regards the character of the terminal product.

In most of the previous investigations of the allantoin excretion in dogs, however, it is difficult or impossible to find out how much of the allantoin in the urine is due to the purine content of the food. The diet is often described only as "liberal" and "mixed." In one investigation by Harding and Young [1919] the quantity and quality of the diet is exactly described (meat and placenta), but the authors make no attempt to calculate how much of the allantoin excreted is due to the exogenous purines. It appears, however, from their tables, when we calculate the excretion of allantoin N in percentage of the total N, that on meat diet there has been an excretion of allantoin N varying between 4.3 and 9 % of the total N excretion. On placental diet the allantoin excretion has been 9-14 % of the total N.

The authors draw attention only to the difference between the output of allantoin on meat diet and on placental diet and conclude from these comparative feeding experiments that the comparatively large amount of arginine present in the placental diet is responsible for the increase in purine metabolism, thus confirming the conclusion of Ackroyd and Hopkins [1915] who in their experiments on rats found that arginine and histidine may serve as substrate for allantoin formation in this animal. Harding and Young do not make any remark on the large excretion of allantoin on meat diet, for which the purine content of the food can only be responsible to a certain limit.

Findings of large amounts of allantoin in dog's urine are very important on account of conclusions drawn from comparative studies of purine metabolism in man and dogs, and based upon the assumption that the allantoin excretion in dogs has the same significance as the uric acid excretion in man. Folin, Berglund and Derick [1924] in their studies of the destruction of intravenously injected uric acid in the dog and man, are aware of the fact that the allantoin excretion in dogs is unreasonably large, though they have no experiments bearing on this phenomenon. They remark (p. 400) that "from the standpoint of tissue metabolism, the finding of several times as much 'endogenous' purine derivatives as in man, in animals whose creatinine excretion is of the same order as that of man, points to some important missing link in the current, accepted concepts. Either the magnitude of purine metabolism in man is hidden by very extensive destruction of purine materials,

or the purine derivatives, including allantoin, found in the urine of other animals have a double origin, as in birds."

In order to obtain experimental evidence for or against the probability of such a double origin of the allantoin and uric acid excretion in dogs, we have made a series of feeding experiments in which we have determined the total N, allantoin and uric acid excretion on protein-free diet, on purine-free protein diet and on high and low protein diet containing known amounts of purines.

The dogs, all mongrels, were from the time they were puppies fed on bread exclusively—no meat.

The urine was collected during 24 hours. The nitrogen was estimated by the Kjeldahl method, uric acid by the method of Folin and Wu [1919], allantoin by the method of Wiechowski [1913].

Table I.

I. *Dog A. Female, weight 9.50 kg. 120 g. fat only.*

Date	Total N g.	Uric acid mg.	Allantoin g.	Uric acid N mg.	Allantoin N g.
4. x.	1.73	Trace	0.85	—	0.30
5. x.	2.56	"	1.00	—	0.35
Average	2.14		0.92	—	0.33

15.5 % of total N

II. *Dog A. 200 g. horse-flesh + 100 g. fat.*

12. x.	5.97	79	1.84	26	0.64
13. x.	5.38	110	1.40	37	0.49
14. x.	5.02	80	1.28	26	0.45
15. x.	5.05	80	1.32	26	0.46
16. x.	5.97	107	1.94	36	0.68
Average	5.47	91	1.55	30	0.54

10 % of total-N

III. *Dog B. Male, weight 8.90 kg. 500 g. horse-flesh.*

10. vi.	15.48	164	2.46	55	0.86
11. vi.	15.88	215	2.10	72	0.74
12. vi.	16.40	302	2.37	101	0.83
Average	15.65	227	2.30	76	0.81

5.6 % of total N

It appears from I that the excretion of allantoin N in the dog on a pure fat diet is 15.5 % of the total N excretion, a percentage which is 15 times as high as in man on a fat diet.

From II and III it is seen that both the allantoin and the uric acid excretion increase when protein is given. Findings of 10 % and 5.6 % of the total N as allantoin N and uric acid N when feeding horse-flesh, which is relatively poor in purines, are extraordinarily high, compared with the values for uric acid N in man in corresponding experiments. Burian and Schur [1900] have determined the purine content of different tissues, and they found [1900, p. 309, Table VIII] in fresh horse-flesh 0.065 % purine N, equal to about 2 % of the total nitrogen. It is thus impossible that the purines in 200 g. of

horse-flesh can be responsible for more than 0.13 g. purine N, and in 500 g. of horse-flesh for more than 0.32 g. purine N (allantoin N + uric acid N) in the urine.

Most of the excreted allantoin and uric acid must therefore in these experiments have another origin than the purine content of the food. Either the break-down of nucleic materials in the body must have increased when feeding horse-flesh, or purines are synthesised from purine-free protein.

In feeding experiments with 700 g. of horse-flesh on two other dogs the excretion of purine derivatives was less than in the previous experiments with less meat, the excretion being on the average 2.7 and 2.5 % of the total N.

Table II.

Dog C. Female, weight 17.9 kg. 700 g. horse-flesh.

Date	Total N g.	Uric acid mg.	Allantoin g.	Uric acid N mg.	Allantoin N g.	Remarks
12. v. and 13. v.	19.9	198	1.34	66	0.47	Urine put together for 2 days. Average for 24 hours
				2.7 % of total N		

Dog E. Male, weight 19.4 kg. 700 g. horse-flesh.

Date	Total N g.	Uric acid mg.	Allantoin g.	Uric acid N mg.	Allantoin N g.	Remarks
12. v. and 13. v.	20.2	167	1.27	56	0.45	Average for 24 hours
				2.5 % of total N		

Three other dogs were fed exclusively with milk and bread *ad libitum*. They had been on this diet for weeks, and there was no change in the diet when they were placed in the metabolic cage. As appears from Table III there is a difference in the excretion of allantoin and uric acid in these dogs which can neither be due to the difference in the quality of the food, nor to the quantity of protein metabolised. The excretion of allantoin and uric acid is greatest in a dog (dog G) which has a smaller excretion of total N than the other dogs. The excretion of purine N is 5.3 % of the total N in this dog, and the total amount is the highest found in any of these experiments. It is evident that the excretion depends upon some other important factor than the existence of certain amino-acids in the proteins supplied.

Table III.

Dog F. Female, weight 14.00 kg. Bread and milk ad libitum.

Date	Total N g.	Uric acid mg.	Allantoin g.	Uric acid N mg.	Allantoin N g.
1. vi.	22.15	248	1.23	83	0.43
				2.33 % of total N	

Dog G. Male, weight 21.5 kg. Bread and milk ad libitum.

Date	Total N g.	Uric acid mg.	Allantoin g.	Uric acid N mg.	Allantoin N g.
3. vi.	18.13	436	2.35	145	0.82
				5.3 % of total N	

Dog H. Male, weight 17.00 kg. Bread and milk ad libitum.

Date	Total N g.	Uric acid mg.	Allantoin g.	Uric acid N mg.	Allantoin N g.
5. vi.	18.14	157	1.25	52	0.44
				2.7 % of total N	

The suggestion of a synthesis in mammals of purines from purine-free protein dates from recent years. The starting point for all researches on this problem is the observation of Folin [1905] made 20 years ago, that the endogenous uric acid excretion in man is influenced to a considerable extent by the protein content of the food. Folin found that a change from a protein-rich diet, consisting of eggs and milk, to a protein-poor diet, consisting of cream and starch, might be accompanied by a considerable decrease in the excretion of uric acid—a decrease, however, which was not the same in all individuals.

This observation of Folin was later confirmed by a number of investigators [for lit. see Rose, 1921]. Most of the earlier explanations of this phenomenon are based upon the conception that the nucleins are the sole origin of the uric acid in the urine (increased activity of the digestive organs, stimulation of cellular metabolism by amino-acids).

More recently the suggestion was made that the purines might be synthesised from amino-acids. This suggestion is based upon the work of Ackroyd and Hopkins [1915] previously alluded to. These authors found that the allantoin excretion in young rats decreased 40–50 % when the rats were supplied with purine-free diets deprived of arginine and histidine, but complete in every other respect, and that the allantoin excretion again increased when these amino-acids were added to the food. Tryptophan was without any influence on the allantoin excretion. The authors concluded from their experiments that arginine and histidine were usable raw materials for purine synthesis in the body of the rat.

The experiments of Ackroyd and Hopkins are of the greatest importance. They were the first to show that a synthesis of purines from amino-acids occurs in mammals, a synthesis which until that time was known only with certainty in birds and reptiles.

Harding and Young [1919] have thereafter arrived at a similar conclusion as regards dogs. As previously mentioned these authors found that placenta, which is rich in arginine, produced a much greater excretion of allantoin than did meat.

Quite recently Rose and Cook [1925] in experiments on rats have confirmed the results of Ackroyd and Hopkins as far as the relation of histidine to purine metabolism is concerned. On the other hand, arginine failed to affect the output of allantoin.

As regards our experiments, they permit of the conclusion that in dogs on meat diet purine derivatives are excreted in amounts which are extraordinarily high, and which largely exceed the purine content of the meat, and also that dogs on purine-free diet excrete such large amounts of purine derivatives that a synthesis is most plausible.

The experiments do not however permit conclusions as to which amino-acids may be the raw materials for the synthesis of purines.

The most striking feature in our experiments is that the amount of the synthesised allantoin and uric acid varies in the different dogs on the

same diet. The power of synthesis is most probably common to all dogs, but the extent of synthesis seems to depend more upon individual factors than on the kind of amino-acids in the food.

That the purine metabolism varies, not only in the different species but also in different individuals of the same species, is a fact. This is evident both from the varying amount of excreted endogenous purine bodies and from the varying power to destroy intravenously injected uric acid, as shown by Folin, Berglund and Derick [1924]. As regards the dog, the velocity with which the intravenously injected uric acid is destroyed, according to these authors, is different in different dogs, but never irregular in the same dog.

When we calculate the ratio allantoin : uric acid in our experiments we arrive at a similar conclusion. There is no constant relationship between these substances in the urines of these dogs. Hunter and Givens [1914] have introduced the term "uricolytic index" to indicate this relationship. The term expresses the percentage of allantoin N on the sum of uric acid N plus allantoin N. In normal dogs Hunter, Givens and Guion [1914] found an index of 98. In studies of the uric acid metabolism in Dalmatian dogs S. R. Benedict [1916, 1917] found that these dogs eliminate more uric acid in proportion to allantoin and that accordingly the index is smaller, varying between 24 and 62.

In other mammals Hunter and co-workers [1914] have found that the index varies between 79 and 98, but is constant in each species, indicating that among mammalia there exist several distinct types of purine metabolism.

The validity of this index as a physiological constant, however, is rendered doubtful, when the "uricolytic index" in our experiments (Table IV) is considered.

Table IV.

Animal (dog)	Uricolytic index (average)	Diet
A	95	200 g. horse-flesh, 100 g. fat
B	91	500 g. horse-flesh
C	87	700 g. horse-flesh
E	89	700 g. horse-flesh
F	84	Bread and milk
G	85	Bread and milk
H	89	Bread and milk

It appears from this table that the "uricolytic index" varies in all these dogs, the highest index being 95, the lowest 84.

It seems therefore as if the oxidation of uric acid also varies individually in the different dogs.

These observations of a varying purine metabolism in animals of the same species support our opinion that the suggested synthesis depends on *individual factors*, which we are inclined to conceive as persisting ontogenetic dispositions.

By studying the comparative biochemistry of the purine metabolism one gets the impression that individual factors play a predominating role.

The varying excretion of "endogenous" uric acid in different human beings, the varying concentrations of uric acid in the blood, the great uric acid excretion in the Dalmatian hound compared with the usual excretion in other dogs, and the varying allantoin and uric acid excretion in different dogs point to individual dispositions, which probably persist from embryonic life and are inheritable, and are such inheritable dispositions as might characterise a family or a race.

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